Alexandrium spp.

Description: *Alexandrium* spp., *Gymnodinium catenatum,* and *Pyrodinium bahamense* var. *compressum* are species of dinoflagellate that produce toxins known as saxitoxins. Several of these toxic species are found along the west coast of North America (including Alaska), the northeast U.S. coast, and in the Canadian maritime provinces.

Toxin Produced: Saxitoxin
Saxitoxin and its derivatives block voltage dependent sodium channels inhibiting nerve conduction and can lead to respiratory paralysis.

Syndrome: Paralytic Shellfish Poisoning (PSP)
Paralytic shellfish poisoning from eating contaminated (PSP) produces symptoms that are mainly neurological with in 30 minutes shellfish. In mild exposures, symptoms include tingling sensations or numbness, headaches, fever, rash, and dizziness. In severe cases symptoms include muscular paralysis, pronounced respiratory difficulty, choking sensation, and death through respiratory failure which may occur within 24 hours.

Distribution: **West Coast:** Alaska to mid-California coastal waters
East Coast: Newfoundland to Connecticut coastal waters

Accomplishments: 1994-present

1994: ***Receptor Assays for Domoic Acid and PSP Toxins***
New receptor-based assays for domoic acid and PSP toxins have been found to be useful for detecting toxins in toxic algae, shellfish, crab hepatopancreas, and the serum of exposed humans and animals. These high capacity assays are formatted to contain 96 data points on a 3 x 4" filter card to provide rapid, reliable results and detect all toxin congeners in a manner quantitatively proportional to their toxicity. These assays are anticipated to be used in dock side testing and confirmation of marine toxin exposure in humans and marine animals. *Contact: Greg Doucette*

1995: ***Detection of Domoic Acid and PSP Toxin Activity in Algae and Animals***
New rapid and inexpensive receptor-based assays for domoic acid and PSP toxins have been found to be reliable for detecting toxins in toxic algae, shellfish, crab hepatopancreas, and the serum and urine of exposed humans and animals. These assays have been validated against HPLC analytical methods and mouse bioassay. National reference laboratories within the European Community have requested that NMFS offer training workshops on implementing the receptor assays and expressed a desire to initiate collaborative testing programs. These assays are anticipated to be used in dockside testing of shellfish and confirmation of marine toxin exposure in seafood consumers. *Contact: Greg Doucette*

1996: ***Production of PSP Toxins by Bacteria***
Recent studies of PSP toxigenesis suggest that both dinoflagellates, as well as certain bacteria associated with these algae, are capable of synthesizing PSP toxins. This has been addressed using both laboratory and field based studies. Reintroduction of bacteria isolated from toxic, but not nontoxic strains of *Alexandrium tamarense* was determined to increase PSP production in axenic *Alexandrium* cultures. Field studies conducted in collaboration with the Department of Fisheries and Oceans (Canada) during a red tide bloom in the lower St. Lawrence River estuary determined that bacteria grown from size excluded isolates

produced PSP toxins. Continuing investigations will focus on how bacterial-algal interactions influence PSP toxigenesis. *Contact: Greg Doucette*

Development of Reporter Gene Assay for Marine Toxins

A new assay technology has been developed for algal toxins. Reporter gene assays have been established using the c-fos response element linked to the coding region for firefly luciferase and this approach has been published (Analytical Biochemistry). This method is very effective for measuring brevetoxins, PSP toxins and ciguatoxins. The method has a particularly high sensitivity for ciguatoxins and should permit a high capacity monitoring of the toxin in small (<1 g) finfish samples. *Contact: John Ramsdell*

1997: ***Passage of PSP Toxins Through the Food Web***

The PSP receptor binding assay (corroborated by HPLC analyses) is being used to study toxin transfer in Gulf of Maine food webs. Work to date has shown that PSP toxins move preferentially from their algal producers (*Alexandrium* spp.) into the larger size fractions of the zooplankton grazing community dominated by large copepods, even though these animals were not numerically dominant. Toxin-accumulating copepods could provide a direct trophic linkage for vectorial intoxication and possible mortality of plankitvorous fish as well as endangered whales which are known to feed upon these copepods. This project is in collaboration with Dr. D. Anderson (WHOI) and Dr. J. Turner (U. Mass. Dartmouth). *Contact: Greg Doucette*

Collaborative Testing of Receptor Assays for Marine Toxins

Receptor based assays for PSP, ASP, NSP, and CFP have been developed and laboratory validation completed in the past four years. These assays are now ready to be tested corroboratively in formal interlaboratory trails. The first of these trails, testing the assay for NSP in lysters, has been initiated as an AOAC Peer Verified Method trial, which will be completed in FY1998. *Contact: Fran Van Dolah*

2000: ***Accumulation of PSP Toxins in Zooplankton Grazers***

Algal toxins are well-known to undergo trophic transfer and accumulation in marine food webs, causing intoxication of upper-level consumers such as fish, sea birds, and marine mammals. Work in collaboration with investigators at U. Mass Dartmouth and the Woods Hole Oceanographic Institution has focused on the tracking movement of PSP toxins from their algal producers into the associated zooplankton grazer assemblage. Through receptor assay-based analysis of algal and zooplankton size fractions from Massachusetts Bay, MA, we determined that PSP toxins did, indeed, accumulate in the grazers and that toxicity was disproportionately concentrated in the larger zooplankton size fractions (200-500 : m, > 500 : m). Interestingly, these size fractions, frequently dominated by large copepods, comprised only a small portion of total zooplankton abundance. These larger toxin-accumulating copepods could thus provide a direct trophic linkage for PSP intoxication of marine mammals such as baleen whales, which are known to preferentially feed upon these grazers. *Contact: Lisa Hollen*

Recent Advances in and Applications For a PSP Receptor Binding Assay

Several years ago we described a high throughput receptor binding assay for PSP toxins and its use for detecting toxic activity in shellfish and algal extracts. We have since increased the assay efficiency through application of microplate scintillation technology (4h turn around time), and have validated use of 11-[^{3}H]-tetrodotoxin as an alternative radioligand to the [^{3}H]-saxitoxin conventionally employed in the assay. Efforts are now focused on identifying the applications for which the receptor assay can provide data comparable to the more time consuming, technically demanding HPLC analysis of PSP toxins. We have compared the results of both methods for toxic dinoflagellates, field samples of *Alexandrium* spp. and its associated zooplankton grazers, as well as contaminated human fluids from a PSP outbreak. In general, receptor-based STX equiv. values were highly correlated and in close quantitative agreement with those produced by HPLC. While the receptor binding assay does not provide toxin composition data

obtainable by HPLC, it does represent a robust and reliable means of rapidly assessing PSP-like toxicity in laboratory and field samples. Moreover, this assay should be effective as a screening tool in suspected cases of PSP intoxication. *Contact: Greg Doucette*

Assay Validation and Technology Transfer

As part of the U.N. sponsored technology transfer program on red tides in SE Asia, we conducted a training workshop on receptor assays in Manila, Philippines in December 1999. The workshop was attended by 14 particiants from 7 SE Asian countries. In addition, this year we hosted two individuals associated with this program for extensive receptor assay training in the laboratory: Ms. Cecilia Conaco, of the University of the Philippines (October-Nov 1999) and Ms. Mei Mei Ch'ng, of University of Malaysia (March – August 2000). We will host up to 3 additional personnel from participating nations during FY 2001. The program will then carry out a round robin interlaboratory comparison trial between participating nations in 2002. A receptor assay training workshop was held in May at CCEHBR to transfer this technology to representatives of two state regulatory agencies interested in its potential as a replacement for the mouse bioassay: California Dept. of Health and Florida DNR *Contact Fran VanDolah*

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